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TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Drug-resistant trypanosome isolates populations in dogs in Enugu North Senatorial Zone, Southeastern Nigeria

Chukwunonso Francis Obi[1] · Michael Ikenna Okpala[1] · Ikenna Onyema Ezeh[1] · Amaechi Onyeabor[2] · Romanus Chukwuduruo Ezeokonkwo[1]

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Abstract

African animal trypanosomosis is an important wasting and endemic protozoan disease causing morbidities and mortalities in animals in the sub-Saharan Africa. Currently, chemotherapy is the widely used method of African animal trypanosomosis control, especially in dogs in the sub-Saharan Africa. However, their efficacy is threatened by the emergence of drug-resistant trypanosomes owing to their extensive use and misuse over several decades amongst other factors. Thus, this study focused on the trypanocidal sensitivity and characterization of *Trypanosoma* species isolated from dogs in Enugu North Senatorial Zone (ENSZ), Southeastern Nigeria. *Trypanosoma brucei* ($n=44$) and *T. congolense* ($n=4$) isolated from naturally infected dogs in ENSZ, Southeastern Nigeria, between January and August 2016 were subjected to single dose test to assess their sensitivity to diminazene aceturate (DA) and isometamidium chloride (ISM). Subsequently, DA and multidrug-resistant isolates were further subjected to DA multi-dose test and CD_{50} was determined and was used to characterize the drug-resistant trypanosomes. Clones were derived from a randomly selected multidrug-resistant isolate and their sensitivity also assessed. 100% and 83.3% of *T. congolense* and *T. brucei* respectively were resistant to the trypanocides. Amongst the drug-resistant isolates, 50%, 16.7%, and 33.3% were resistant to DA, ISM, and both trypanocides respectively with CD_{50} ranging between 11 and 32.34 mg/kg. Drug-resistant trypanosomes were characterized into highly resistant ($CD_{50}=11–24.99$ mg/kg) and very highly resistant ($CD_{50}=>25$ mg/kg) trypanosome isolates. Clones also expressed high levels of resistance to both DA and ISM with CD_{50} values between 35.58 and 38.85 mg/kg. Trypanocidal resistance was, thus, confirmed and appears to be widespread in dogs in ENSZ, Southeastern Nigeria. The adoption of an integrated trypanosomosis control strategy in ENSZ is most desirous.

Keywords *Trypanosoma* sp. · Diminazene · Isometamidium · Drug resistance · Dogs · Southeastern Nigeria

Introduction

In the sub-Saharan Africa, African animal trypanosomosis control methods usually employed include tsetse control, use of trypanotolerant livestock, and chemotherapy (Holmes et al. 2004). The use of trypanotolerant livestock and some tsetse control strategies are fraught with some challenges (Obi et al. 2020). Though the development and implementation of tiny targets has provided some real advantages in vector control (Rayaisse et al., 2020), chemotherapy remains the most widely used trypanosomosis control strategy especially in dogs (Holmes et al. 2004; Akpa et al. 2008). It has been estimated that over 50 million doses of trypanocides are used annually and this is projected to increase annually considering the large number of livestock at risk of the disease (McDermott et al. 2003; Holmes et al. 2004).

Notwithstanding the consistent demand for trypanocides in Africa, most pharmaceutical companies are reluctant to invest in the development and licensing of new animal trypanocides citing low total value ($30 million) of African market as an excuse (Holmes et al. 2004). This increases

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✉ Chukwunonso Francis Obi
Chukwunonso.obi@unn.edu.ng; drfrancis.obi@gmail.com

[1] Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

[2] Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria

the pressure on the few existing trypanocides, namely diminazene aceturate and isometamidium chloride, which have been in use for more than six decades. With the liberalization and privatization of veterinary services in Africa including Nigeria, widespread availability of fake, substandard trypanocides and misuse of trypanocides have been reported (Chitanga et al. 2011; Bengaly et al. 2018; Odeniran et al. 2019). The resultant effect is the emergence of resistance to the commonly available trypanocides by trypanosomes (Delespaux et al. 2008a).

Since the first report of Na'isa (1967) in Northern Nigeria, trypanocidal resistance appears to be extending to the countries where trypanosomosis occurs. Increasing incidences of multiple drug resistance to isometamidium and diminazene have also been reported in some African countries (Delespaux et al. 2008a) including Nigeria (Kalu 1995; Anene et al. 1999). Currently, trypanocidal resistance has been reported in about twenty-one African countries including Nigeria (Afewerk et al. 2006). Many more countries are believed to be affected but unreported, probably due to the arduous and expensive nature of trypanocidal resistance detection tests (Mamoudou et al. 2008).

Though trypanocidal resistance was widely reported in many parts of Africa through case reports and few area-wide surveys, paucity of information exists on its occurrence, prevalence, and distribution in Nigeria especially in companion animals such as dogs. Numerous techniques are available for the detection of trypanocidal resistance and have been extensively reviewed by Delespaux et al. (2008a). Molecular tests are rapid and more convenient method of trypanocidal drug resistance (TDR) diagnosis but its practical application is very limited in most African countries due to lack of well-equipped laboratories and skilled personnel. Since in vivo test remains the gold standard for the detection of trypanocidal resistance (Eisler et al. 2001; Vitouley et al. 2011), this study determined the sensitivity of *Trypanosoma* species isolated from naturally infected dogs in Enugu North Senatorial Zone, Southeastern Nigeria to trypanocides as well as characterization of the drug-resistant *Trypanosoma* species using the in vivo tests in mice.

Materials and methods

Experimental animals

Outbred male albino mice weighing 25–30 g and aged 8–10 weeks old were used. They were kept in clean metal cages in the fly-proof Laboratory Animal Unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. The mice were fed proprietary rodent feed and water was provided ad libitum.

Trypanosome field isolates

The trypanosome isolates were *Trypanosoma brucei* ($n=44$) and *T. congolense* ($n=4$) isolated from forty-eight naturally infected dogs in Enugu North Senatorial Zone (ENSZ), Southeastern Nigeria, between January and August 2016. The identification of the trypanosomes was by parasitological and molecular techniques involving ITS-PCR, Tubulin PCR, and DNA sequencing. The trypanosomes were first inoculated in two mice and when the parasitaemia levels reached log parasitaemia 8.1 per ml of blood, blood samples from the parasitaemic mice were used to infect the experimental mice. Thereafter, the trypanosome isolates were maintained in mice by serial passage.

Trypanocides

The drug sensitivity studies were carried out using diminazene aceturate (Diminaze®, Batch No: 12F25, Exp. 06–17, Pantex, Holland) and isometamidium chloride (Securidium®, Lot 274A1, Exp. 02/19, Laprovet, France). Each drug was constituted following manufacturer's guide and administered intraperitoneally to the mice.

Drug sensitivity in mice

The sensitivity of the forty-eight trypanosome isolates to diminazene aceturate and isometamidium chloride was determined using the single dose test according to the protocol described by Eisler et al. (2001). Succinctly, each trypanosome isolate was inoculated into three groups of six mice each. Two groups of mice were treated with 20 mg/kg and 1 mg/kg body weight diminazene aceturate and isometamidium chloride, respectively, 24 h after inoculation with 10^5 trypanosomes (Herbert and Lumsden 1976). The other group served as the control group and was given distilled water. The blood samples of the mice were examined for the presence of trypanosomes twice a week for 60 days observation period. Where two or more out of the six mice in the trypanocide treated groups became parasitaemic within the observatory period, the isolate was considered resistant. Trypanosome isolates were termed multidrug-resistant where the isolates were resistant to both trypanocides. Parasitaemic mice were humanely euthanized and removed from the experiment.

Characterization of drug-resistant trypanosome isolates and cloning of multidrug-resistant trypanosome isolates

Diminazene-resistant and multidrug-resistant trypanosome isolates were further subjected to diminazene-multi-dose test using the protocol described in Eisler et al. (2001) with slight modifications in the doses of diminazene aceturate. 60 mg/kg diminazene in the protocol described in Eisler et al. (2001) was replaced with 40 mg/kg due to the observed high level of mortality in the groups of mice treated with 60 mg/kg of diminazene. CD_{50} of the drug-resistant trypanosome isolates was determined using probit analysis (Peregrine et al. 1991). The drug-resistant trypanosome isolates were characterized on the basis of their CD_{50} into highly resistant (CD_{50} = 11–24.99 mg/kg) and very highly resistant (CD_{50} = > 25 mg/kg) *Trypanosoma* isolates.

One of the multidrug-resistant trypanosome isolate was randomly selected and two clones were derived from it. Succinctly, 3–5 drops of blood containing the selected multidrug-resistant trypanosome isolate were added to a test tube containing 1 ml of phosphate buffered saline glucose and a cloning buffer (20% calf serum). The tube was first centrifuged at 50 *g* for 5 min to separate the cells from the trypanosomes and the supernatant was transferred to another tube and centrifuged at 1000 *g* for 5 min. A drop from the sediment of the second centrifugation was placed on a microscope slide, covered with a cover slip, and examined at × 40 objective lens of the microscope. The level of parasitaemia was quantified (Herbert and Lumsden 1976) and the sediment was further diluted when necessary depending on the number of trypanosomes present, with phosphate buffered saline glucose containing 20% calf serum till one trypanosome was present per drop. Using 1 ml syringe, 0.1 ml of diluted sediment containing one trypanosome was drawn and was further extended with 0.2 ml of diluents (phosphate buffered saline glucose containing 20% calf serum) before intraperitoneal inoculation in mice. Inoculated mice were examined daily from day 3 PI and when parasitaemia was detected following wet film examination of tail blood and/or buffy coat technique, drops of blood from the mice were added into a tube containing 1 ml of phosphate buffered saline glucose and 20% calf serum. The procedure above was repeated and sub-inoculation was made in two mice. When the sub-inoculated mice attained log parasitaemia 8.1 per ml of blood, the clones were tagged *Clone 1*. The method was repeated to produce *Clone 2*. The clones and the original isolate were further subjected to both single dose and diminazene-multi-dose tests. The CD_{50} of the clones was also determined.

Statistical analysis

Interpretations of the results of the single and multi-dose tests were made according to Eisler et al. (2001). The sensitivity of the trypanosome isolates to trypanocides was presented in percentages and 95% confidence intervals. Standard probit analysis (Peregrine et al. 1991) was used to determine the CD_{50} of the trypanosome isolates. All statistical analyses were carried out using SPSS version 21 for windows.

Results

Amongst the forty-four *Trypanosoma brucei* isolates screened, twenty-four isolates (54.5%) were sensitive while twenty isolates (45.5%) were resistant to trypanocides (Table 1). Of the twenty resistant *Trypanosoma brucei* isolates, nine (45%) and three (15%) isolates were resistant to diminazene aceturate and isometamidium chloride respectively while eight isolates (40%) were resistant to both trypanocides (multidrug-resistant). All the *T. congolense* isolates were resistant to the trypanocides, with three isolates being multidrug-resistant and one isolate resistant to isometamidium chloride alone. Of the forty-eight *Trypanosoma* isolates subjected to single dose test for their sensitivity to

Table 1 Sensitivity of *Trypanosoma species* isolates obtained from naturally infected dogs in Enugu North Senatorial Zone, Enugu State, Nigeria

	Trypanosoma brucei	*Trypanosoma congolense*	OVERALL	
	Number of isolates (%)	Number of isolates (%)	Number of isolates (%)	95% Confidence interval
Sensitive isolates	24 (54.5)	0 (0)	24 (50)	0.352–0.648
Resistant isolates	20 (44.5)	4 (100)	24 (50)	0.352–0.648
DAR isolates	9 (45)	0 (0)	12 (50)	0.314–0.686
ISMR isolates	3 (15)	1 (25)	4 (16.7)	0.061–0.365
MDR isolates	8 (40)	3 (75)	8 (33.3)	0.178–0.534

KEY: *DAR*, diminazene aceturate resistant isolates; *ISMR*, isometamidium chloride resistant isolates; *MDR*, multidrug-resistant isolates

diminazene aceturate and isometamidium chloride, twenty-four (50%; 95% CI = 0.352–0.648) were sensitive to both diminazene aceturate and isometamidium chloride while the other twenty-four (50%; 95% CI = 0.352–0.648) were resistant (Table 1). Amongst the resistant trypanosome isolates, twelve trypanosome isolates (50%; 95% CI = 0.314–0.686) were resistant to diminazene aceturate only, four trypanosome isolates (16.7%; 95% CI = 0.061–0.365) were also resistant to isometamidium chloride alone while eight trypanosome isolates (33.3%; 95% CI = 0.178–0.534) were resistant to both diminazene aceturate and isometamidium chloride (multidrug-resistant).

The clones of the randomly selected multidrug-resistant trypanosome isolate (*VTH 22*) expressed high levels of resistance to both diminazene aceturate and isometamidium chloride (Table 2) analogous to the original isolate. The CD_{50} of *VTH 22 Clone 1* and *VTH 22 Clone 2* was 38.85 mg/kg and 35.58 mg/kg respectively and was about 1.3 times higher ($P < 0.05$) than that of the original isolate (26.23 mg/kg).

The CD_{50} of the drug-resistant trypanosomes ranged between 11 and 32.34 mg/kg. Sixteen drug-resistant trypanosome isolates with CD_{50} ranging between 11 and 24.99 mg/kg were categorized as highly resistant *Trypanosoma* isolates (Table 3) while four isolates with CD_{50} above 25 mg/kg were categorized as very highly resistant *Trypanosoma* isolates (Table 3).

Discussion

The results of the current study have shown that drug-resistant trypanosomes are prevalent in dogs in ENSZ, Southeastern Nigeria. Anene et al. (1999) also detected drug-resistant trypanosome isolates in some dogs presented to the University of Nigeria Veterinary Teaching Hospital (UNVTH), Nsukka between August 1992 and March 1994. Canine trypanosomosis treatment failures were prevalent in most veterinary clinics in Southeastern Nigeria (Anene et al. 1999), particularly the UNVTH, Nsukka, which is a reference veterinary hospital in the Southeastern Nigeria and could be attributed to drug-resistant trypanosomes. Drug-resistant trypanosomes have also been detected in pigs in Southeastern Nigeria (Eze et al. 2015) and in cattle in Africa including Nigeria (Sinyangwe et al. 2004; Delespaux et al. 2008b; Mamoudou et al. 2008; Mulandane et al. 2017; Tchamdja et al. 2017; Odeniran et al. 2019).

The high occurrence of drug-resistant trypanosomes may be connected to the practices of most dog owners, breeders, and sellers in the study area. Trypanocides and antibiotics are usually administered to every batch of dogs (both apparently healthy and sick dogs) procured for sale by the dog sellers. Also, most dog owners and breeders only present

Table 2 Diminazene aceturate resistance profile of *VTH 22* and clones derived from *VTH 22* following multi-dose test

	No. of mice relapsed					*No. of days to relapse					CD_{50} (mg/kg)	
Clones	1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	CD_{50}	95% CI
VTH 22	6	6	6	3	2	9	20	28	35	56	26.23[a]	14.50–84.16
VTH 22 Clone 1	6	6	6	5	3	5	17	22	31	53	38.85b[b]	22.10–161.94
VTH 22 Clone 2	6	6	6	4	3	6	19	25	33	51	35.58	20.34–704.22

[ab]Different superscripts across the column represent significant difference at $P < 0.05$

KEY:

*Number of days post treatment when at least two mice had relapsed in treatment groups

CD_{50}, curative dose for 50% of the infected population

CI, 95% confidence interval

Table 3 Characterization of drug-resistant trypanosomes into highly resistant (CD_{50} = 11–24.99 mg/kg) and very highly resistant (CD_{50} = > 25 mg/kg) *Trypanosoma* isolates in ENSZ

Isolates	Trypanosome specie	No. of mice relapsed					*No. of days to relapse					CD_{50} (mg/kg)		
		1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	CD_{50}	95% CI	Classification
Orba 17	*T. congolense*	6	6	4	2	0	9	21	32	60	NR	13.77	6.31–22.66	Highly resistant
Orba 19	*T. brucei*	6	6	5	2	1	10	14	25	56	35	17.84	8.79–33.05	Highly resistant
Orba 4	*T. brucei*	6	6	5	3	2	8	10	20	35	49	23.73	12.10–110.45	Highly resistant
Orba 10	*T. brucei*	6	6	5	3	1	10	13	25	42	56	20.09	10.44–42.91	Highly resistant
Iba 4	*T. brucei*	6	6	4	3	2	13	22	35	56	60	21.08	10.13–89.16	Highly resistant
Iba 5	*T. brucei*	6	5	4	2	1	12	19	28	35	49	13.03	5.87–32.97	Highly resistant
Iba 8	*T. brucei*	6	6	4	3	2	8	17	21	14	49	21.08	10.13–89.16	Highly resistant
Iba 9	*T. brucei*	6	6	4	3	1	10	21	28	42	56	17.66	8.53–39.91	Highly resistant
VTH 4	*T. brucei*	6	6	3	4	2	10	14	21	35	60	21.88	10.14–157.41	Highly resistant
VTH 5	*T. brucei*	6	5	4	3	2	8	17	28	49	58	19.46	8.14–178.43	Highly resistant
VTH 6	*T. congolense*	6	6	5	2	1	12	21	30	42	57	17.84	8.79–33.05	Highly resistant
VTH 8	*T. brucei*	6	5	4	2	0	12	19	26	58	NR	11	5.31–21.92	Highly resistant
VTH 9	*T. brucei*	6	6	5	3	0	11	19	26	60	NR	17.49	10.42–29.64	Highly resistant
VTH 13	*T. brucei*	6	6	5	3	1	9	13	21	42	60	20.09	10.44–42.91	Highly resistant
VTH 20	*T. congolense*	6	5	4	3	1	10	23	35	35	49	15.35	6.91–51.09	Highly resistant
VTH 21	*T. brucei*	6	5	4	4	1	7	17	22	56	60	18.41	8.24–100.26	Highly resistant
Orba 9	*T. brucei*	6	6	6	5	2	10	9	23	28	49	32.34	19.68–220.14	Very highly resistant
Iba 1	*T. brucei*	6	6	6	4	2	6	17	28	49	60	29.27	17.36–121.46	Very highly resistant
VTH 11	*T. brucei*	6	6	6	4	2	9	15	28	42	42	29.27	17.36–121.59	Very highly resistant
VTH 22	*T. brucei*	6	6	6	3	2	9	20	28	35	56	26.23	14.50–84.16	Very highly resistant

KEY: *NR*, no relapse; *Number of days post treatment when at least 1 or 2 mice had relapsed in treatment groups with 1 or more relapsed mice, respectively; *CD_{50}*, curative dose for 50% of the infected population; *CI*, 95% confidence interval

their dogs to the veterinary clinics after self-medication with trypanocides have failed. Most times, these trypanocides are improperly reconstituted, weight of dogs incorrectly estimated, and low doses administered over several days to the dogs. These factors could have contributed to the observed high prevalence of trypanocidal resistance. Generally, large-scale dosing, high treatment frequency, lack of proper diagnosis, uncontrolled use of trypanocides, and the exposure of parasites to sub-therapeutic drug concentrations, resulting from under-dosing, were considered the major causes of increasing drug resistance throughout Africa (Anene et al. 2001; Holmes et al. 2004).

In Nigeria and most sub-Saharan tsetse infested countries, trypanocides represent about 40–50% of the total animal health/drugs market (Sutcliffe et al. 2014; Kingsley 2015). This offers an enormous and tempting target for the importation and sales of substandard, counterfeit, or unregistered trypanocides. The privatization and liberalization of veterinary services in Africa including Nigeria has increased the influx of substandard, fake, and unregistered trypanocides into the Nigerian pharmaceutical markets especially by non-professional stakeholders (Kingsley 2015). Numerous generic forms of trypanocides with striking variations in their quality are also available in most Nigerian markets including those in ENSZ. Studies have shown that many of the trypanocides circulating in sub-Saharan African markets, including Nigeria, have quality issues (Anene et al. 2006; Teko-Agbo et al. 2008; Bengaly et al. 2018). This is further compounded by the absence of internationally acceptable quality control standards for trypanocides to ensure the quality and concentration of active ingredients or their content in the finished veterinary medicinal products (Sutcliffe et al. 2014). Though, it cannot be concluded that the high prevalence of drug-resistant trypanosomes reported in the present study is an indication of this, it is a possibility that cannot be ignored.

Trypanocide-linked mutations in the trypanosomes and/or the increased prevalence of drug-resistant trypanosomes in the vectors may have also contributed to the high occurrence of drug-resistant trypanosomes detected in the current study. Chitanga et al. (2012) reported a high prevalence of diminazene-resistant *T. congolense* in areas without a history of drug exposure due to the widespread presence of diminazene-linked mutations in the gene coding for P2-type purine transporters. Trypanocidal drug sensitivity is not affected by tsetse transmission as stability of drug resistance can persist over a period of time (Geert and Holmes, 1998). Thus, increase in vector density and resultant increased transmission intensity of trypanosomes to animals in trypanocidal resistant endemic areas increases the risk of spread of drug-resistant trypanosomes. The preponderance of trypanosome infected *Glossina tachinoides* and *G. palpalis palpalis* has been reported in the Southeastern Nigeria (Madubunyi 1987; Onyekwelu et al. 2017). However, the sensitivities of trypanosomes in infected tsetse in the study area to trypanocides have not been investigated. Simo et al. (2020) reported the occurrence of diminazene aceturate-resistant *T. congolense* in tsetse flies trapped in Yoko in the Centre Region of Cameroon.

Half of the resistant trypanosome isolates in ENSZ were found to be resistant to diminazene aceturate alone. This finding was not surprising and could be linked to the popularity, affordability, and widespread use of diminazene aceturate over isometamidium chloride in the treatment and control of canine trypanosomosis and babesiosis. A study on the knowledge, attitudes, and practices of farmers, animal health attendants, livestock sellers, and veterinarians on trypanosomosis and trypanocides revealed that diminazene aceturate was the most abused trypanocide probably because of its availability, affordability, and pocket-friendly costs. This exposes the problems clinicians face in the successful treatment and management of trypanosomosis in the area. The predominance of trypanosomes resistant to diminazene aceturate over that of isometamidium chloride were also reported by Delespaux et al. (2008b) in Eastern Zambia, Mamoudou et al. (2008) in Adamaoua Region of Cameroon, and Mulandane et al. (2017) in Zambezia Province of Mozambique. Resistance of the trypanosome isolates to isometamidium chloride alone was the least prevalent as only four isolates were isometamidium chloride-resistant. In most African countries, high rate of isometamidium chloride resistance has been reported (Sinyangwe et al. 2004; Tchamdja et al. 2017). The low prevalence rate of isometamidium chloride-resistant trypanosome isolates could be attributed to the fact that isometamidium chloride is not the trypanocide of choice for the treatment of canine African trypanosomosis and it is more expensive than diminazene aceturate, and thus, rarely used in the study area.

The occurrence of multidrug-resistant isolates in ENSZ was also established in this study. Similar findings were also reported in some dogs presented to the UNVTH, Nsukka between August 1992 and March 1994 by Anene et al. (1999), cattle in Burkina Faso (McDermott et al. 2003), Eastern Province of Zambia (Sinyangwe et al. 2004), Ethiopia (Afewerk et al. 2000; Moti et al. 2012), Cameroon (Mamoudou et al. 2008), Mali (Mungube et al. 2012a), Togo (Tchamdja et al. 2017), and Mozambique (Mulandane et al. 2017). Drug-resistant trypanosome field isolates may consist of a pan-mictic population of trypanosomes resistant to a single drug or a homogenous population of multi-resistant trypanosomes or a mixture of both (Mamoudou et al. 2008). As such, two clones derived from a randomly selected multidrug-resistant isolate (*VTH 22*) to verify if the observed multidrug resistance phenotype was clonal revealed high levels of resistance to both trypanocides analogous to the original

isolate, with their CD_{50} about 1.3 times higher ($P < 0.05$) than that of the original isolate. Codjia et al. (1993), Mulugeta et al. (1997), and Afewerk et al. (2000) also reported high levels of resistance to the trypanocides by trypanosome clones following their study in Ethiopia. The import of this finding is that the exploitation of sanative pair as a strategy to surmount trypanocidal resistance in ENSZ is under serious threat and may very soon become ineffective.

Drug-resistant trypanosomes were characterized on the basis of their CD_{50} into highly resistant ($CD_{50} = 11–24.99$ mg/kg; $n = 16$) and very highly resistant ($CD_{50} = > 25$ mg/kg; $n = 4$) trypanosome isolates. The conventional dose for the treatment of canine trypanosomosis due to *T. brucei* and *T. congolense* is 7 mg/kg and 3.5 mg/kg body weight, respectively (Holmes et al. 2004). Thus, to achieve therapeutic cure, the highly resistant and very highly resistant trypanosome isolates will require about 1.6–3.5 and 3.5–5 times the conventional dose (7 mg/kg for *T. brucei*), respectively. Anene et al. (2006) reported the occurrence of a drug-resistant *T. brucei* isolate in a dog which was successfully treated using 45 mg/kg body weight of diminazene aceturate in the study area. Though the curative dose for dogs cannot be extrapolated from mice treatment studies (Eisler et al. 2001), these results have provided an in-depth information on the levels of drug resistance of the individual trypanosome isolates.

The findings of the present study have wide epidemiological implications. Firstly, considering the high occurrence of drug-resistant canine trypanosomes in the study area, it does imply the circulation of drug-resistant trypanosomes in tsetse flies and other livestock in the study area, given the restricted movement of tsetse flies. Therefore, field studies to evaluate the development of trypanocidal drug resistance in tsetse and other livestock and its impact on livestock productivity in Nigeria are essential. Secondly, trypanocides especially diminazene aceturate and/or the concept of sanative pair may be ineffective at the moment in ENSZ. As such, the use of best bet strategies (Mungube et al. 2012b) involving rational use of trypanocides, treating of sick animals only and after proper diagnosis, boosting of immunity of at risk animals via strategic deworming and administration of feed (Tchamdja et al. 2017) and vitamin supplements, and vector control and quality control of available trypanocides are strongly advocated for the control of animal trypanosomosis.

In conclusion, trypanocidal resistance is prevalent in dogs in Enugu North Senatorial Zone, Southeastern Nigeria. This heightens the impediments to pet rearing, livestock production, and the attainment of the second sustainable development goal (SDG) which seek to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture in the region. Therefore, there is an urgent need for strict monitoring and rational use of trypanocides in the region as well as adoption of the integrated/best bet strategies (Mungube et al. 2012b) of trypanosomosis control.

Author contribution Conceptualization: RCE, CFO, RCE; methodology: RCE, IOE, CFO; formal analysis: CFO; investigation: CFO, MIO, IOE, OA; data curation: CFO; writing — original draft: CFO; writing — review and editing: RCE, IOE, CFO; funding acquisition: RCE; supervision: RCE.

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Declarations

Ethical approval Ethical clearance (FVM-UNN-IACUC-2019–0919) was duly obtained from the University of Nigeria Faculty of Veterinary Medicine Institutional Animal Care and Use Committee. Also, all relevant procedures and rules for the ethical care and use of laboratory animals were duly followed.

Conflict of interest The authors declare no competing interests.

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